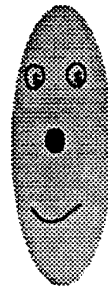


MICROBIOLOGY:THE BIO IN BIOREMEDIATION

BIOREMEDIATION - THE ESSENTIALS



BIOREMEDIATION

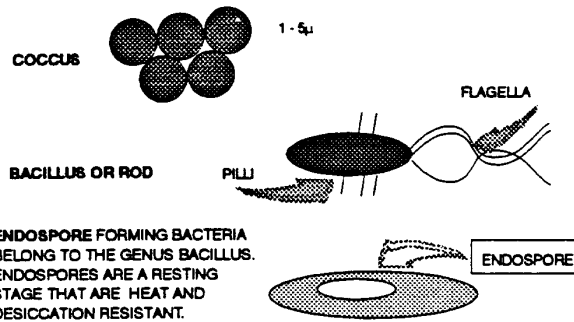


Hydrocarbonaceous profitis

WHAT ARE BACTERIA?

TRADITIONALLY BACTERIA WERE DEFINED ON THE BASIS OF STRUCTURAL, NUTRITIONAL, AND ECOLOGICAL PROPERTIES. TODAY WE HAVE A BETTER UNDERSTANDING OF THE BIOCHEMICAL BASIS FOR THESE DISTINCTIONS. HOWEVER, THE TRADITIONAL CLASSIFICATIONS ARE STILL USED.

DEFINING BACTERIA BASED ON THEIR SHAPE



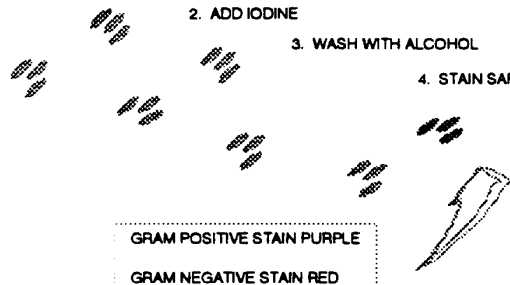
USING THE GRAM STAIN TO DEFINE BACTERIA

1. STAIN CRYSTAL VIOLET

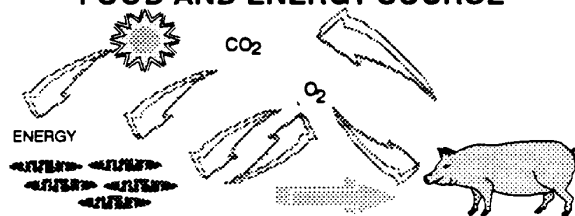
2. ADD IODINE

3. WASH WITH ALCOHOL

4. STAIN SAFFARIN

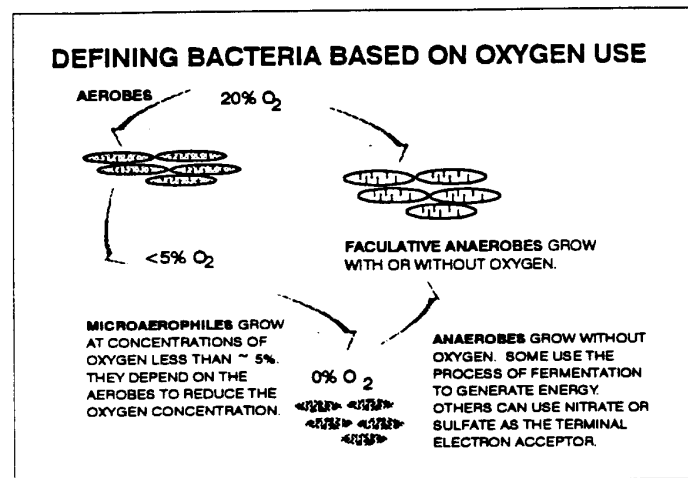
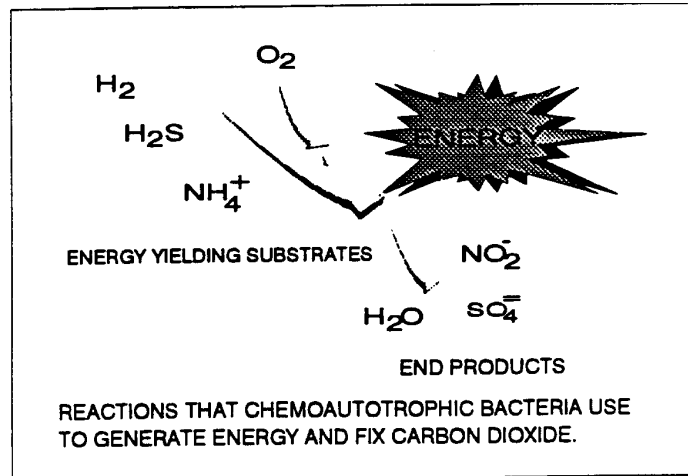


DEFINING BACTERIA BASED ON FOOD AND ENERGY SOURCE



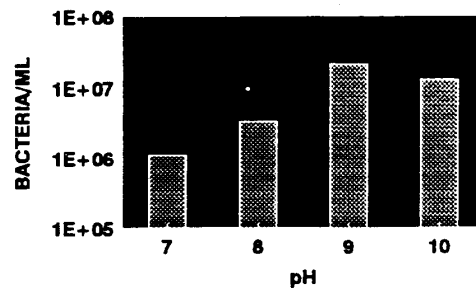
AUTOTROPHS BLUE GREEN ALGAE AND PLANTS USE SUNLIGHT AS AN ENERGY SOURCE AND CARBON DIOXIDE AS THE SOURCE OF CARBON. SOME BACTERIA USE HYDROGEN, AMMONIA, SULFIDE, AND OTHER REDUCED INORGANIC CHEMICALS AS A SOURCE OF ENERGY.

HETEROTROPHS USE THE ORGANIC MATTER PRODUCED BY AUTOTROPHS AS A SOURCE OF FOOD AND ENERGY. HETEROTROPHS INCLUDE HUMANS AND BACTERIA.



BACTERIA DEFINED ON THE BASIS OF THE OPTIMUM GROWTH pH

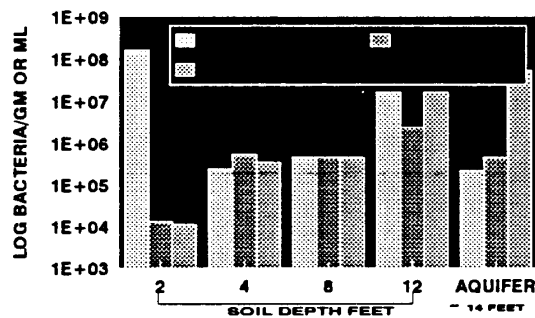
- ACIDOPHILES 1 - 4
- NEUTROPHILES 6 - 8
- ALKALIPHILES 9 - 12



BACTERIA FROM AN ALKALINE DESERT SOIL AT MCB 29 PALMS
ENUMERATED IN MEDIUM OF INCREASING pH ILLUSTRATE THEIR
ADAPTATION TO THE ALKALINE CONDITIONS IN THE SOIL

BACTERIA DEFINED BASED ON THEIR REQUIREMENTS FOR SODIUM

- TERRESTRIAL - Na NOT REQUIRED
- MARINE - REQUIRE Na
- HALOPHILES REQUIRE > 2.5M NaCl



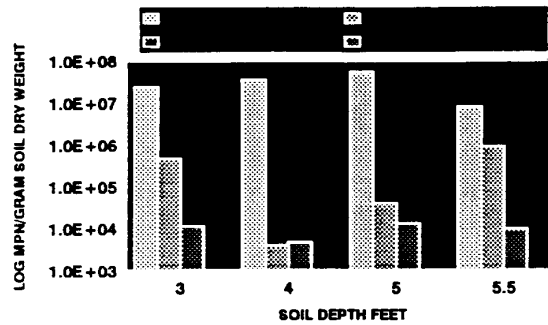
BACTERIA IN SOIL AND WATER FROM A SALINE AQUIFER WERE ENUMERATED IN
NUTRIENT BROTH AND FALLON BROTH PREPARED WITH SODIUM (Na+) OR
POTASSIUM (K+) AS THE MAJOR CATION. THE RESULTS SHOW A DISTINCT SHIFT
IN THE BACTERIAL POPULATION AS THE ENVIRONMENT SHIFTS FROM
TERRESTRIAL (NEAR SURFACE) TO MARINE (SALINE AQUIFER).

BACTERIA DEFINED ON THE BASIS OF THEIR OPTIMUM GROWTH TEMPERATURE

■ PSYCHROPHILES < 15C

■ MESOPHILES 15 - 45C

■ THERMOPHILES > 45C



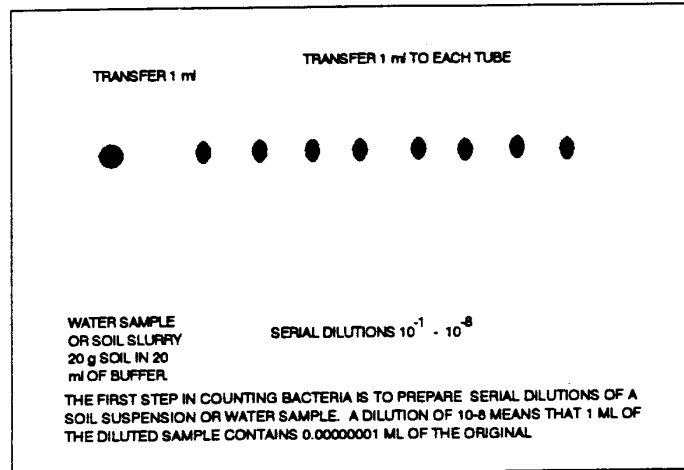
ENUMERATION OF HETEROTROPHS AND HYDROCARBON DEGRADERS IN SOIL TAKEN AT THE INDICATED DEPTHS FROM A BIOPILE THAT HAD SPONTANEOUSLY WARMED TO 50C. BACTERIA WERE COUNTED IN MEDIUM INCUBATED AT 25C OR 45C. NO HYDROCARBON DEGRADERS GREW IN MEDIUM INCUBATED AT 45C.

HOW WE COUNT BACTERIA

■ PLATE COUNTS

■ FLUORESCENCE COUNTS

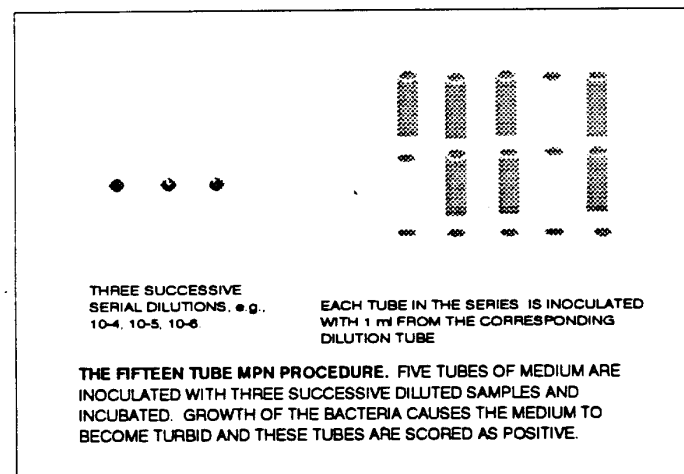
■ MOST PROBABLE NUMBER (MPN)



THE CONTENTS OF THE DILUTION TUBES ARE USED TO INOCULATE TUBES OF MEDIUM - TO DETERMINE THE MPN OR SPREAD ON MEDIUM SOLIDIFIED WITH AGAR TO PERFORM A PLATE COUNT.

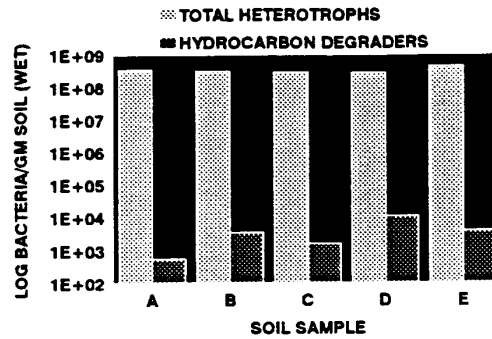
HETEROTROPHS ARE ENUMERATED USING NUTRIENT BROTH WHICH IS A MINERAL SOLUTION SUPPLEMENTED WITH YEAST EXTRACTS AND OTHER COMPLEX SOURCES OF VITAMINS, AMINO ACIDS, LIPIDS AND PROTEINS.

HYDROCARBON DEGRADERS ARE ENUMERATED IN A BASAL SALTS SOLUTION SUPPLEMENTED WITH HYDROCARBON MIXTURES, e.g., JP-5 OR SINGLE HYDROCARBONS, e.g., NAPHTHALENE.

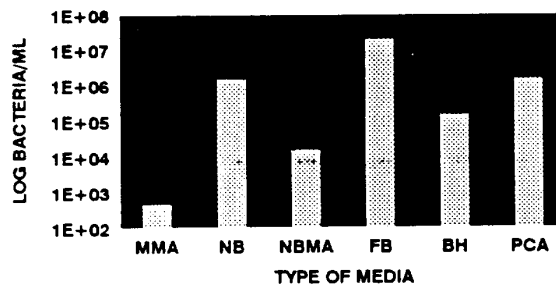


CALCULATING THE NUMBER OF BACTERIA

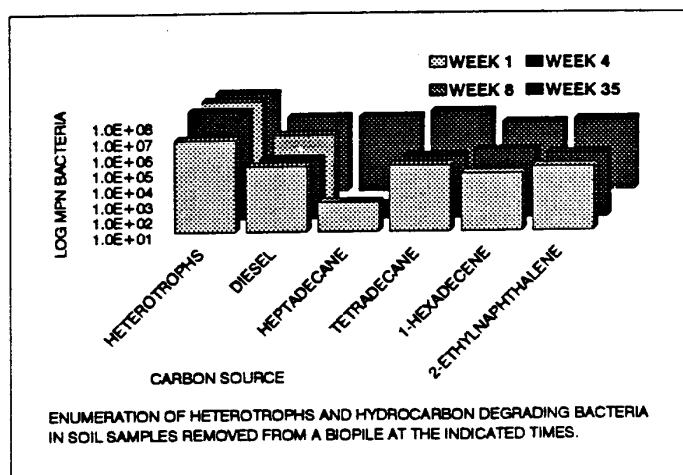
- THE PATTERN OF POSITIVE TUBES 4-3-0 CORRESPONDS TO AN MPN OF 2.7. MPN VALUES ARE OBTAINED FROM STANDARD TABLES.
- THIS NUMBER IS DIVIDED BY THE LOWEST DILUTION OF THE THREE SUCCESSIVE DILUTIONS USED TO INOCULATE THE MPN TUBES - WHICH IN THIS EXAMPLE WAS 10^{-4} .
- THUS THE NUMBER OF BACTERIA IN THE ORIGINAL WATER OR SOIL SUSPENSION IS
- $2.7/10^{-4} = 2.7 \times 10^{-4}$ BACTERIA/ML
- IF THE TUBES CONTAIN NUTRIENT BROTH THIS NUMBER IS REPORTED AS THE NUMBER OF HETEROTROPHS. IF THE TUBES CONTAIN DIESEL OR SOME OTHER HYDROCARBON THIS NUMBER IS REPORTED AS THE NUMBER OF HYDROCARBON DEGRADERS.



ENUMERATION OF HETEROTROPHS AND AND JP-5 DEGRADING BACTERIA IN FIVE DIFFERENT SOIL SAMPLES COLLECTED FROM UNCONTAMINATED SOIL NEAR MCB KANEOHE BAY, HI.

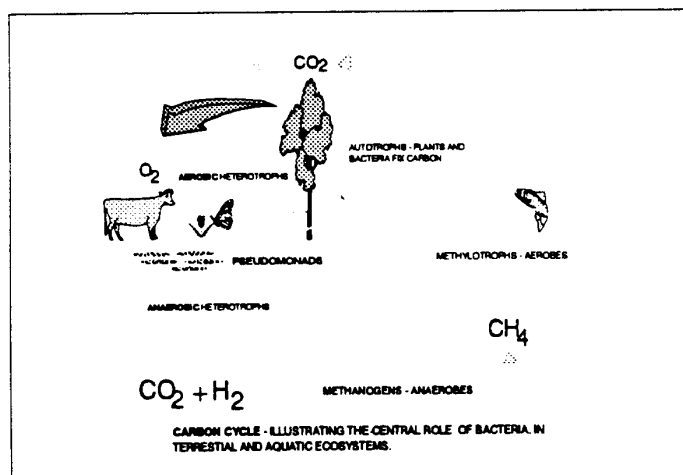


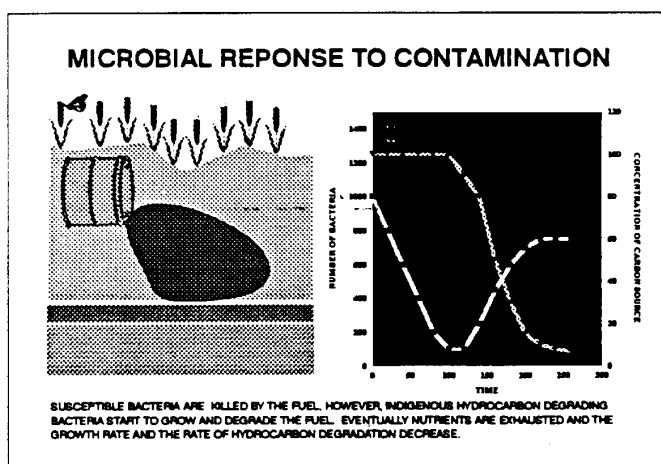
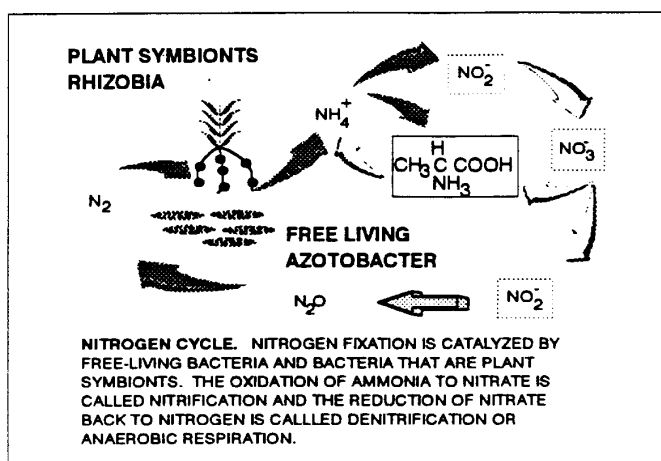
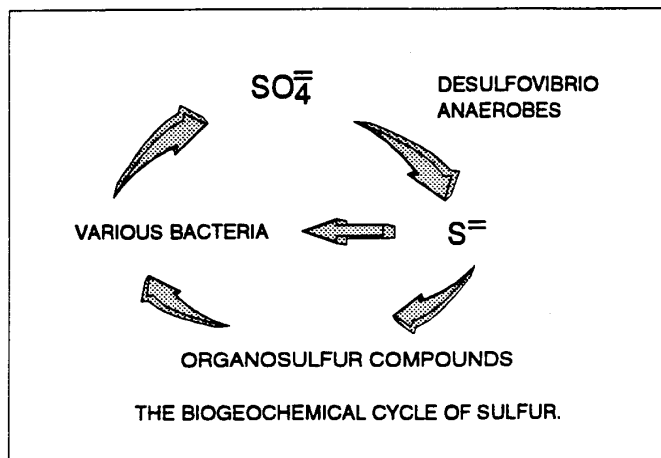
ENUMERATION OF BACTERIA IN SOIL SAMPLES FROM FALLON NAS IN SIX TYPES OF NUTRIENT BROTH. MINIMAL MEDIUM A SUPPLEMENTED WITH YEAST EXTRACT AND CASAMINO ACIDS (MMA), NUTRIENT BROTH (NB), NUTRIENT BROTH PREPARED WITH MMA SALTS SOLUTION (NBMA), FALLON BROTH (FB), BUSHNELL-HASS (BH) AND PLATE COUNT AGAR (PCA).



DEFINING BACTERIA ON THE BASIS OF THEIR ECOLOGICAL FUNCTION

BACTERIA RECYCLE ELEMENTS BETWEEN INORGANIC AND ORGANIC FORMS. ON A GLOBAL SCALE THIS RECYCLING OF THE ELEMENTS IS REFERRED TO AS THE BIOGEOCHEMICAL CYCLES.





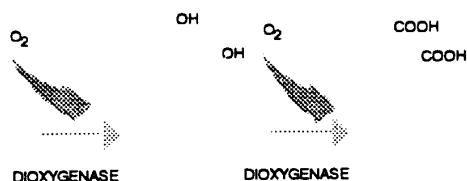
WHAT IS BIODEGRADATION?

BIODEGRADATION IS THE USE OF HYDROCARBONS AND OTHER CONTAMINANTS AS A SOURCE OF FOOD AND ENERGY BY BACTERIA THAT POSSESS THE NECESSARY ENZYMES. AS A RESULT, HYDROCARBONS ARE CONVERTED TO CARBON DIOXIDE, WATER, AND COMPONENTS OF THE BACTERIAL CELL.

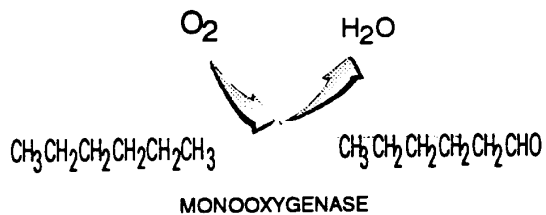
BIOREMEDIATION UTILIZES THE BIODEGRADATIVE CAPABILITIES OF BACTERIA TO CLEAN-UP CONTAMINATED SITES.

BIOSTIMULATION ADDS OXYGEN AND OTHER NUTRIENTS TO CONTAMINATED SITES TO STIMULATE BIODEGRADATION.

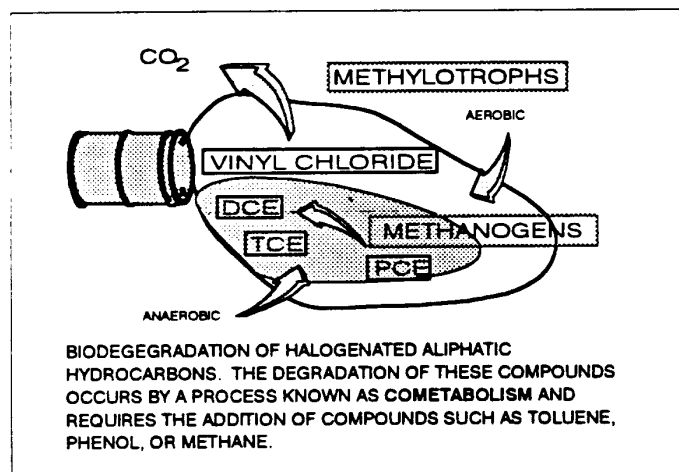
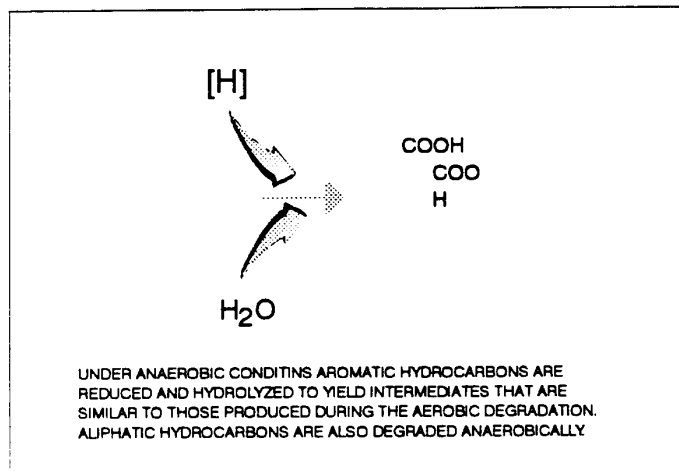
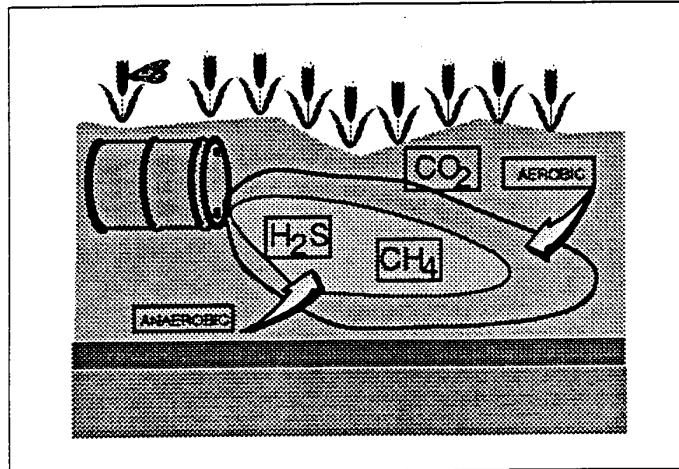
BIOAUGUMENTATION IS THE ADDITION OF HYDROCARBON DEGRADING BACTERIA TO A CONTAMINATED SITE.

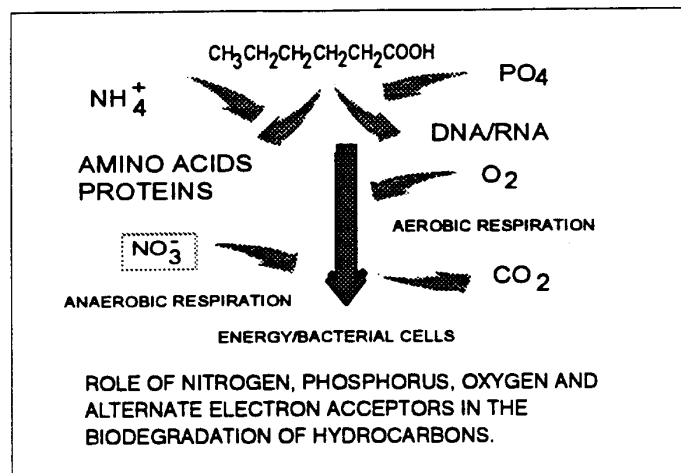
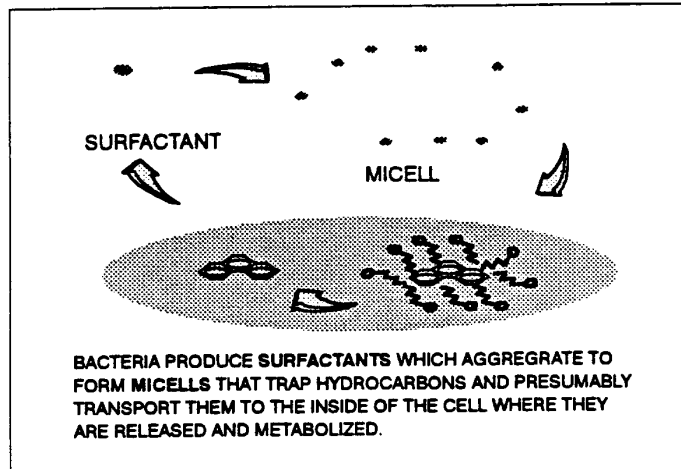


OXIDATION OF AROMATIC HYDROCARBONS UNDER AEROBIC CONDITIONS REQUIRES ENZYMES REFERRED TO AS DIOXYGENASES. THE STEPWISE OXIDATION YIELDS MOLECULES THAT THE BACTERIA CAN CONVERT TO AMINO ACIDS AND OTHER BUILDING BLOCKS OR FROM WHICH THEY CAN EXTRACT ENERGY.

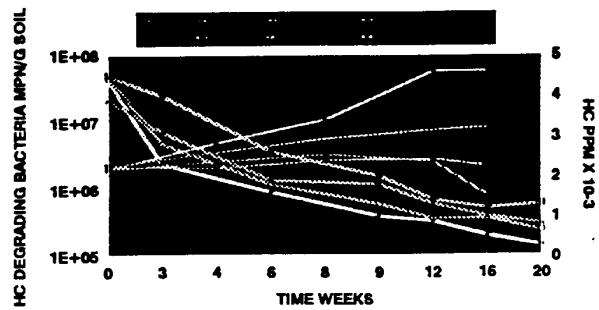


OXIDATION OF ALIPHATIC HYDROCARBONS UNDER AEROBIC CONDITIONS REQUIRES ENZYMES REFERRED TO AS MONOOXYGENASES. THE STEPWISE OXIDATION YIELDS MOLECULES THAT THE BACTERIA CAN CONVERT TO AMINO ACIDS AND OTHER BUILDING BLOCKS OR FROM WHICH THEY CAN EXTRACT ENERGY.

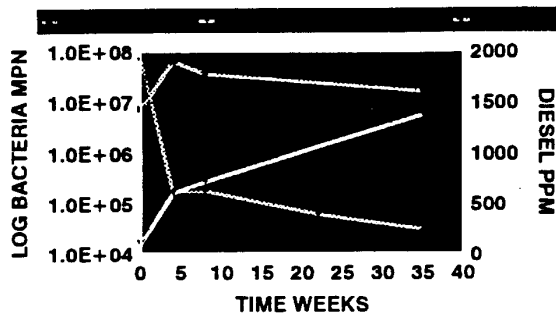




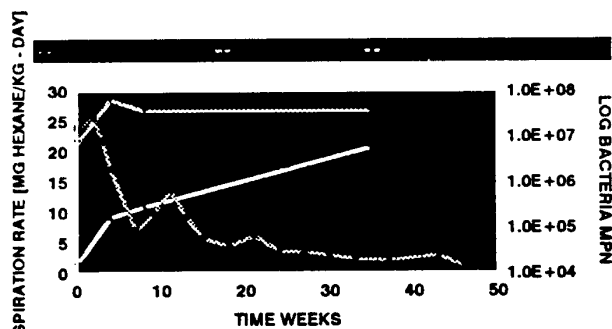
TREATABILITY STUDIES AND FIELD RESULTS



RESULTS OF A TREATABILITY STUDY IN WHICH SOIL COLUMNS WERE: (1) LEFT STATIC (2) AERATED (3) AERATED WITH MOISTURE ADDED (4) AERATED WITH MOISTURE AND NUTRIENTS ADDED. RESULTS SHOW THE NUMBER OF HYDROCARBON DEGRADING BACTERIA AND THE DIESEL (HC) CONCENTRATION.



CONCENTRATION OF RESIDUAL FUEL AND ENUMERATION OF HETEROTROPHS AND HYDROCARBON DEGRADERS IN A BIOPILE USED TO TREAT SOIL CONTAMINATED WITH AGED DIESEL.



ENUMERATION OF HETEROTROPHS AND HYDROCARBON DEGRADERS IN A BIOPILE USED TO TREAT SOIL CONTAMINATED WITH AGED DIESEL. ALSO SHOWN IS THE RESPIRATION RATE WHICH IS USED TO MONITOR THE PROCESS.

WHAT CONTROLS THE RATE OF DEGRADATION?

- OXYGEN
- WATER
- NITROGEN
- PHOSPHOROUS
- OTHER NUTRIENTS
- ACCESS TO/AVAILABILITY OF THE FUEL
- NUMBER OF BACTERIA
- TEMPERATURE